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חוק הפטנטים, התשכ"ז-1967  
PATENTS LAW, 5727-1967

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בקשה לפטנט  
Application for Patent

אני, (שם המבקש, מענו – ולבי נס פואוג – מקום התאגורתו)  
1 (Name and address of applicant, and, in case of a body corporate, place of incorporation)

Aaron Lewis  
18/14 Neve Shalom  
Jerusalem 93707

אaron Lewis  
18/14 Neve Shalom  
Jerusalem 93707

שםהה הוא: Owner, by virtue of being the inventor בעל אמצעה מכח  
of an invention, the title of which is:

(בבביה)  
(Hebrew)

Deconvolving Far-field Optical Images Beyond the Diffraction Limit by using Scanned Probe Optical and Non-optical Data as the Constraint in Mathematical Constraint Algorithms

(באנגלית)  
(English)

hereby apply for a patent to be granted to me in respect thereof.

מבקש בזאת כי ניתן לי עליה פטנט.

| * בקשה חילוק<br>Application for Division  |                | * בקשה טפטן מוקדם<br>Application for Patent of Addition |                          |                                    | * דרישת דין קורימת<br>Priority Claim |  |  |
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| dated<br>dated  | dated<br>dated | dated<br>dated  | dated<br>dated           | dated<br>dated                     | dated<br>dated                       |  |  |
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| הןן למסירת הדעתות ומסמכים בישראל<br>Address for Service in Israel                                     |                |   |                          |                                    |                                      |  |  |
| 18/14 Neve Shalom<br>18/14 Neve Shalom<br>Jerusalem 93707   |                |   |                          |                                    |                                      |  |  |

|   |
|---|
| חתימת המבקש<br>Signature of Applicant                     |
| 18/14 Neve Shalom<br>18/14 Neve Shalom<br>Jerusalem 93707 |

היום 14 בחודש פברואר 1994 שנה  
This 14 of February 1994

לשימוש הלשכה  
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Deconvolving Far-field Optical Images Beyond the Diffraction Limit by  
using Scanned Probe Optical and Non-optical Data as the Constraint in  
Mathematical Constraint Algorithms

## I. Field of the Invention

The field of the invention is the combination of scanned probe microscopic data with far field optical and other images in order to deconvolve these images beyond the diffraction limit.

## II. Background of the Invention

Lens based far-field imaging is limited in the resolution that it can achieve by the characteristics of the lens. In general there are the problems of diffraction of the lens, the problems with aberration of the lens and the problems of out-of-focus radiation. The latter problem is generally partially improved by the use of confocal imaging methodologies or, in optics, also non-linear imaging techniques are useful, while the solution of the former problems are partially addressed by measuring the point spread function of the lens and then using computer deconvolution to remove these effects from the image. Even the latter problem can be addressed without confocal or non-linear imaging by considering both the in-focus and the out-of-focus point spread function and using deconvolution routines to try and eliminate these effects. Numerous algorithms have been devised to address these problems of computer deconvolution of far-field imaging data but none are completely successful and none of them have the ability to carry the far-field image to the realm beyond the diffraction limit as defined for example by the Rayleigh criterion, which is approximately 1/2 of the wavelength of the radiation that is being used or for visible 500 nm light this is 250 nm.

In terms of deconvolution algorithms, a powerful mathematical approach is based on the use of constraints. For example, in deconvolving a far-field image a good constraint would be to define with high precision the cell membrane of a cell that is stained with a dye and is being imaged by a lens. By precisely defining the position of a cell membrane or a portion of the cell membrane we can precisely define where the staining in the image is confined and beyond which point or points there is no staining and its associated optical phenomenon. Such a constraint would give many deconvolution algorithms a powerful advantage. Nonetheless, even though the idea is mathematically a powerful concept [Carrington et al, *Science* 268, 1483 (1995)], it is seriously limited in far-field optics by the inability to obtain a constraint that is better than the optical resolution.

## III. State of Prior Art

No one has previously attempted to incorporate such near-field optical data and other scanned probe microscope data such as that which is obtained from atomic force microscopy to the problem of providing constraints in the deconvolution of far-field optical and other far-field imaging techniques.

## IV. Summary of the Invention

A method for deconvolving far-field optical images beyond the diffraction limit by using near-field optical and other scanned probe imaging data such as that which can be obtained from atomic force microscopy on a region of the far-field data set in an integrated and inter-digitated way in order to provide powerful and new constraints for the deconvolution of far-field data sets to resolutions beyond the diffraction limit of the lens that is being used or in the case of non-linear optical imaging or other microscopies to resolutions beyond that which is achievable with these microscopies.

## V. Description of the Invention

The present invention incorporates data that has never been incorporated previously to try and resolve these issues of far-field imaging. This data comes from near-field optical microscopy and its scanned probe cousins such as atomic force imaging (AFM) and presents the far-field microscopist with constraints that will allow for improving dramatically the far-field imaging of all forms of far-field microscopy both linear and non-linear optical microscopy and even those microscopies that use particles rather than electromagnetic radiation. It also allows for using one form of scanned probe microscopy to deconvolve another and thus improve the scanned probe microscopic resolution before this data is used in this invention as described below.

For such an approach it is essential to obtain the near-field optical or other scanned probe imaging data in a way that is fully integrated with the far-field data set. In one embodiment of this invention, in which far-field optical microscopic data is to be deconvolved, to achieve the required full integration of the data sets one useful approach is to use a charge coupled device (CCD) to record the far-field optical image. The near-field optical and AFM or other scanned probe data can be related directly to the exact digital pixel of the far-field data that is corresponding to the associated scanned probe pixel. Furthermore, it is additionally useful if the scanned probe data sets that are to be used in the deconvolution can be obtained in simultaneous channels. This can be done for example with a tip that is multifunctional such as a tip that is both a subwavelength light source and an AFM sensor [K. Lieberman, et al, Rev. Sci. Instr. 67, 3567 (1996)] that can be used in contact or near-contact with a surface of the specimen that is being imaged.

However, even before the far and near-field imaging process is begun, the image of the subwavelength light source of known dimension can on the CCD is a measure of the point spread function (PSF), which is the lens function that convolutes with the functional representation of the sample to give the blurred image that is the far-field image with its associated diffraction and other problems mentioned in the Background section of this patent. Alternately the convolution effect of the lens can also be achieved if a known high resolution sample is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens. Obviously, if the imaging task is fluorescence then the high resolution test object will have to be similarly fluorescent. thus the first task in this method is the PSF of the lens.

The next step is to record with the CCD the far-field image. Subsequently, super-resolution optical data can be recorded at specified points on the sample surface. Examples of such points can include: an exact point at which the optical contrast in a sample terminates, i.e. defining the edge of a sample to much better than the optical resolution if the far-field image is an optical image; or defining the x, y and z point or voxel at which there is a contrast change and relating this point to another point of contrast change in the sample. In terms of the relation of two points of contrast change in the sample, these could be at different planes as defined by the lens in the far-field. The scanned probe data, through the simultaneously recorded AFM, can provide not only the xy separation of the two points but also the Z separation at a resolution that is better than any optical approach such as confocal microscopy.

The exemplary constraints listed above or for that matter any constraints from scanned probe technology have never been used in this cross-fertilization mode

with far-field optical microscopy or for that matter any far-field microscopic technique. In addition such cross-fertilization has not been used between scanned probe techniques, i. e. to use near-field optical data as a constraint at deconvolving atomic force microscopy data or the reverse. With regard to this latter mode of deconvolution there is quite a bit of synergism between, for example, the near-field optical and the AFM since the functional dependence of the decay of the effect, as a function of probe sample separation, is near-exponential for the near-field optical and occurs over a much shorter distance for the simultaneously recorded AFM technique. In essence then the scanned probe microscopy synergism can first be used to improve the scanned probe microscopy data and then that data can be applied as a constraint to the far-field microscopy deconvolution in question. It is also important to note at this point, that, the order of the procedures listed in this section is not a critical part of the invention, any combination of the order of the steps or partial combination of steps constitutes this invention. For example, if the near-field optical data is not used to deconvolve to higher resolution the atomic force microscopy data, it, and the atomic force microscopy data could still be used to deconvolve the far-field data.

The CCD mentioned above is a most useful method to obtain the digitized image of the far-field but this can also be accomplished with confocal microscopy. In the case of far-field optical microscopy it should be noted that there could be innovative ways to record the confocal data without any confocal aperture. For example, if there is a film of material that could produce a non-linear optical signal known as second harmonic generation (SHG) this could be used in an innovative way as part of this invention to record a confocal image. In this case the light from the plane of focus would be focused by the lens onto a film, such as a plastic film of purple membrane that produces SHG [Z. Chen et al, *Applied Optics* 30, 5188 (1991)], with an intensity that is higher than from any other plane in the sample that is being focused by the lens. Thus such a film could be used to replace the confocal pinhole by using a film that would produce a second harmonic signal only where there is a point of light from the plane of focus in the sample and thus such a film would act as a parallel filter for light from the plane of focus in the sample. This could be used together with an appropriate filter after the film to remove the fundamental wavelength that was illuminating the sample and only passes the SHG to the detector which could be a CCD rather than a single channel detector that is normally part of a confocal set-up. This could be done with SHG or other non-linear optically active films.

#### **VI. Advantages Over Prior Art**

Scanned probe microscopy data has not been used as a constraint in mathematical constraint algorithms to deconvolve far-field optical images. In addition, the use from multi functional scanned probe microscopy of one parameter, such as near-field optical data, to deconvolve another parameter such as atomic force microscopy data has also not been applied. The advantage over prior art arises from the increase in spatial resolution that this approach achieves.

#### **VII. Applications**

Methodologies for increased spatial resolution always open new doors in science and technology. Look at the revolution that was caused by the introduction of the electron microscope.

To test the essence of this invention we have performed a calculation on a model far-field optical data set. In Figure 1 there are four parts. Starting from the top of the figure is a model object. When the object is imaged by a lens with a known point spread function the blurred image, which is the second from the top in Figure 1, results. When this image is recorded by a CCD there is further blurring due to the pixel character of the CCD. The last image in this figure is a result of a standard deconvolution algorithm without the imposition of the type of constraints that is central to this invention in which a new approach to provide constraints is described. In Figure 2 the same object, lens, and CCD are assumed but in the deconvolution algorithm just 4 points are given with the resolution of near-field optics. These points are, in going from left to right in the model object, the first, second, third and fourth alterations in contrast. The results of using such constraints is seen in the vastly improved quality of the deconvolved image.

**Figures**

Figure 1 A model object (first image) imaged by a lens (second image), recorded on a CCD (third image) and deconvolved without constraints.

Figure 2 The same as Figure 1 but the deconvolution was performed with constraints.

FIGURE 1

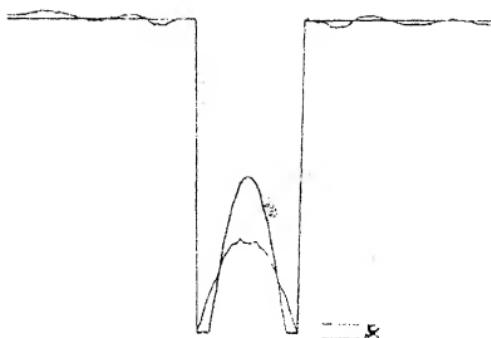
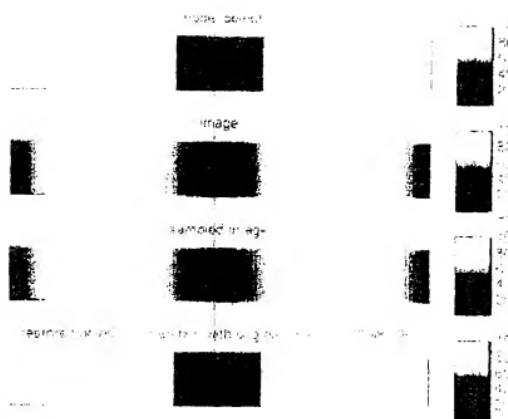
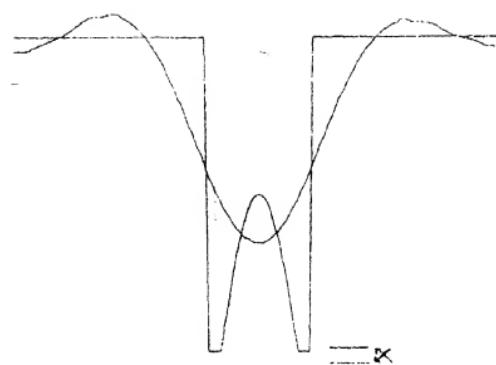
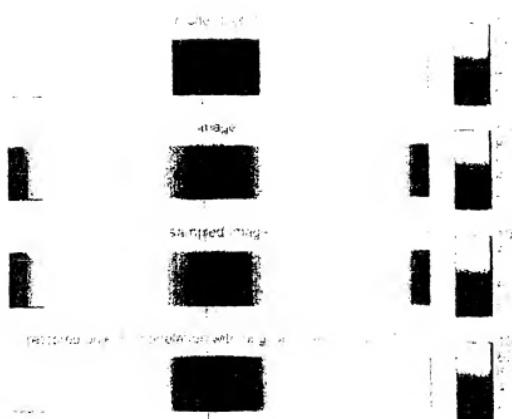


FIGURE 2



### Claims

1. A method for deconvolving far-field optical microscopic images to a level of resolution that has never been achieved previously by introducing scanned probe microscopy data in an interdigitated, integrated fashion with confocal or charge couple device imaging fashion such that every pixel or voxel from the super-resolution scanned probe microscopy data set is directly related to an image pixel or voxel in the far-field data set and then using the scanned probe data as a mathematical constraint to deconvolve the far-field image
2. A method that uses multifunctional scanned probe microscopy data so that one scanned probe microscopy parameter can be deconvolved by integrating data from another such as, but not excluding other combinations, deconvolving an atomic force microscopy image with a near-field optical microscopy image or vice versa.
3. The method as in claim 1 in which the procedure in claim 2 is used in combination with the method in claim 1.
4. A method to obtain the point spread function, which is a crucial step to the deconvolution described in these claims, by using a known high resolution sample either fluorescent or non-fluorescent, which is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens and is representative of an accurate point spread function.
5. A method to obtain a digitized image of the optical far-field in addition to simple confocal or charge coupled device imaging that can be used for integrated near-field and far-field imaging that uses a film of material that can produce a non-linear optical signal known as second harmonic generation in which light from the plane of focus of the lens would be focused by the far-field lens onto a film, such as plastic purple membrane films, with an intensity that is higher than from any other plane in the sample that is being focused by the lens and thus such a film could be used to replace the confocal pinhole in confocal imaging by using a film that would light up only where there is a point of light from the plane of focus in the sample and thus such a film would act as a parallel filter for light from the plane of focus in the sample with this light being imaged through a filter for the fundamental illuminating frequency onto a charge coupled device.
6. A device that collects in an integrated, correlatable fashion that as a result allows for deconvolving far-field optical microscopic images to a level of resolution that has never been achieved previously by introducing scanned probe microscopy data in an interdigitated, integrated fashion with confocal or charge couple device imaging such that every pixel or voxel from the super-resolution scanned probe microscopy data set is directly related to an image pixel or voxel in the far-field data set and then using the scanned probe data as a mathematical constraint to deconvolve the far-field image
7. A device that collects in an appropriate integrated fashion multifunctional scanned probe microscopy data so that one scanned probe microscopy parameter can be deconvolved by integrating data from another such as, but not excluding other combinations, deconvolving an atomic force microscopy image with a near-field optical microscopy image or vice versa.

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7. A device as in claim 5 that also has the capabilities of claim 6

8. A device to obtain the point spread function, which is a crucial step to the deconvolution described in these claims, by using a known high resolution sample either fluorescent or non-fluorescent, which is imaged and the error between the real and the ideal image is represented as a blurring function introduced by the lens and is representative of an accurate point spread function.

9. A device to obtain a digitized image of the optical far-field in addition to simple confocal or charge coupled device imaging that can be used for integrated near-field and far-field imaging that uses a film of material that can produce a non-linear optical signal known as second harmonic generation in which light from the plane of focus of the lens would be focused by the far-field lens onto a film, such as plastic purple membrane films, with an intensity that is higher than from any other plane in the sample that is being focused by the lens and thus such a film could be used to replace the confocal pinhole in confocal imaging by using a film that would light up only where there is a point of light from the plane of focus in the sample and thus such a film would act as a parallel filter for light from the plane of focus in the sample with this light being imaged through a filter for the fundamental illuminating frequency onto a charge coupled device.

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